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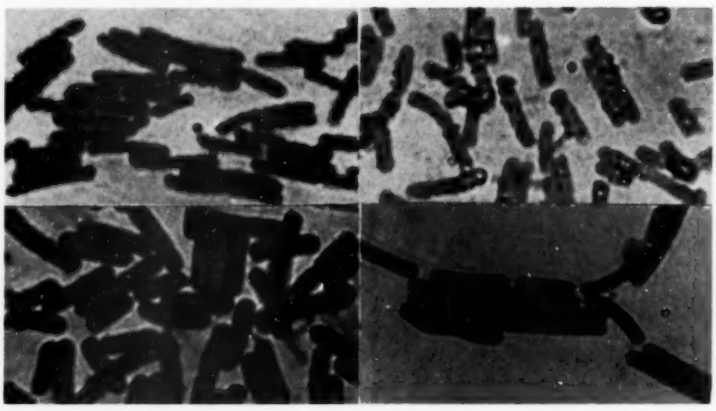

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E D I T O R I A L

A PLEA FOR UNITY

ONCE again the pendulum has swung and in Washington we have the first Republican Administration in twenty years. The quality of the new appointees and the swiftness with which the responsibilities of government have been assumed, speak well for the future and for the administrative ability of our new President.

Other great executives in our country's history have faced serious problems but President Eisenhower is confronted with issues which dwarf all past problems into insignificance. The whole world is involved in a struggle and the free world looks to us for leadership. The presidency of the United States is no longer a national responsibility; it is truly international in character. In this connection it is fortunate that General Eisenhower is not the typical military man, concerned primarily in winning battles, but that he has a breadth of vision and great human understanding. Already he has endeared himself to millions by that common touch and personal warmth which radiate from him wherever he goes. His insight into human relations is an invaluable asset, for his will be the task of getting many divergent factions—social, political and national to work together for the common good.

Already there is evidence that some great changes will be made in the approach used in accomplishing desired ends. For many years, now, class warfare has been a useful political tool and it has been used so much that Americans have become largely organized into selfish, snarling gangs each jockeying for a better position with the cost to the nation of no concern whatsoever. Self-sacrifice for the common good, once an American virtue, is now looked upon as sheer folly and enlightened self-interest is used to describe the most base and selfish motives.

The honest, forthright approach which President Eisenhower appears to favor is certain to disturb certain pressure groups which have been accustomed to getting their wishes by "deals" and secret agreements. The policy of containment, which is purely defensive, seems certain to be replaced by a positive approach to the problems

of the free world. This should relieve that sense of frustration which has overtaken most Americans for we are not inclined as a people to refuse to face our perils. Our friends abroad may find it difficult to adjust to a new policy of expecting our financial aid to be matched by concrete results—not necessarily to ourselves but in advancing the cause of freedom and strength. We must, ourselves, be prepared for certain unpleasant facts long obscured by the preceding several administrations. Principal among these is that our economy for many years has been supported artificially by war and deficit spending. It is very doubtful that our present standard of living could be maintained if we should return to a peacetime economy, balance the budget and reduce our national debt. Yet, these things *all* honest and moral Americans must not only do but devoutly hope and pray for. Those who would not sacrifice toward this end are guilty of preferring to enjoy luxuries bought at the expense of human blood—that of our boys and others equally precious to someone, somewhere.

Peace and a high standard of living are not incompatible by any means, but the transition period is almost certain to be fraught with difficulties and these should be expected. Some of these were already apparent just prior to the Korean conflict and they would have become much worse had it not been for the stimulus (and more deficit spending) which this gave our economy. We do not believe the Truman Administration deliberately or knowingly used Korea as a means of postponing economic difficulties but it did so nevertheless.

The purpose of this editorial is not only to wish the new Administration well but also to ask all who call themselves Americans and freedom loving people everywhere to give it their full support. The issues that face us are so great that partisan politics, pressure from farm blocs, labor or industry all must be subjugated willingly that we may succeed.

This may be the last chance for the survival of those institutions which give man freedom and human dignity. America, not through choice but by stark necessity, must assume world leadership and must perform much better than our past record suggests. Great civilizations in the past have succumbed and in every case it was through internal moral decay. Luxury and ease while threatened by a harsh and cruel foe was the downfall of Greece and Rome. It took centuries to regain what was lost. Unless western civilization recognizes this danger we ourselves may be engulfed just as surely.

Every American, regardless of his political party, his job, his color or creed must be willing to forego personal gain and support this government in the difficult days ahead. The stakes are high—the survival of our civilization and our way of life. President Eisenhower is no magician nor does he have supernatural powers. His strength and his success will be based on the strength and character of his people.

L. F. Tice

THE EFFECT OF CERTAIN ANTISEPTICS ON CARYOPHANON LATUM Peshkoff

By Louis Gershenfeld and Gow T. Lam *

Introduction

THE observations of the changes in the bacterial cell itself, brought about by the action of germicides was demonstrated with the electron microscope (24) (25).

Many workers have investigated the mode of action of various disinfectants. Ehrlich (9) studied the effect of the arsenicals on trypanosomes and revealed that tervalent or quinquevalent arsenicals were oxidized or reduced respectively to the corresponding arsenoxide, and that the arsenoxide in turn reacted with the reduced sulfhydryl groups of the cell. Fildes (11) observed that mercuric salts acted similarly to arsenoxide, both precipitating the proteins of the cells by combining with the sulfhydryl groups. McCalla (21) observed that ions of Na^+ and K^+ were only weakly absorbed and were not toxic in dilute solution, whereas ions such as Hg^{++}, Ag^+ and H^+ were strongly absorbed and were toxic in dilute solutions. Silver nitrate is irritating, astringent, and corrosive when in contact with tissues; and in a 1:10,000 concentration will inhibit the growth of most bacteria (28). Baker *et al* (1) studied the bactericidal action of detergents and found that the effect of detergents were not limited to interference with the enzyme systems of the cell but depended upon a nonspecific effect on the cell membranes and proteins, resulting in alteration of the permeability of the cell wall. Phenol has been described as to its ability to react with proteins to form insoluble proteinates (22), thus its toxicity has been attributed to its ability to denature proteins.

Caryophanon latum was first isolated from cow dung in 1940 by Peshkoff (4) (33). This organism is a large Gram-negative bacillus, arranged in trichomes (29), having an average size of 3 microns in width and from 15 to 20 microns in length, and it is peritrichously flagellated. The organism occurs singly and mostly in

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pairs attached end to end, some in chain formation. Each organism (trichome) contains many striations as observed in water mounts under a compound microscope. There are many granules within each trichome ranging from 0.2 to 0.3 micron in diameter which are located near the cytoplasmic membrane.

This investigation was undertaken to study the effects of certain antiseptics on *Caryophanon latum* by microscopic technics. This organism was chosen for this investigation because of its size. Since it is one of the largest bacteria known, the internal structures are readily observed with the usual microscopic procedures.

Materials

The following materials were employed in this study:

A. *Antiseptics:*

1. Phenol, aqueous solutions of 1:20 and 1:100 concentrations.
2. Resorcinol, 1 per cent aqueous solution.
3. Mercury bichloride, 1:1,000 aqueous solution.
4. Sodium arsenite, 1 per cent aqueous solution.
5. Silver nitrate, 1 per cent aqueous solution.
6. Sodium fluoride, 1 per cent aqueous solution.
7. Sodium cyanide, 1:1,000 aqueous solution.
8. Lugol's Iodine Solution (Gram's modification).

B. *Surface active agents:*

1. "Tergitol" Penetrant 4, 1:100 aqueous solution.[1]
2. Tetrosan 60%, 1:100 aqueous solution.[2]

C. *Stains:*

1. Methylene blue, 1:100 aqueous solution (for bacilli).[3]
2. Knaysi's cell-wall stain (19).

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1. Carbide and Carbon Chemical Division, Carbide and Carbon Corp., lot #8 42013.
2. Onyx Oil and Chemical Co., Jersey City, N. J.
3. The Coleman and Bell Co., Norwood, O.

D. *Media:*

Cow dung extract broth and agar media.

The cow dung extract medium was prepared by mixing fresh cow dung with an equal part of tap water in a bottle. The extraneous material was removed by straining through two layers of cheese cloth. The filtrate was dispensed in 50 ml. bottles and in culture tubes.

Cow dung extract agar also was prepared by the addition of 1.5 per cent agar to the filtered material.

Both preparations were sterilized at 15 pounds' pressure (121°C.) for 20 minutes.

E. *Organism:*

Caryophanon latum Peshkoff.

Caryophanon latum used in this investigation was isolated from fresh cow dung. The latter was diluted with tap water in a wide-mouth bottle, covered with a cotton plug and incubated at 20° C. for 24 hours. At the end of the incubation period a loopful of the supernatant was removed and observed under a compound microscope to note if *C. latum* was present. Material containing *C. latum* was streaked on cow dung extract agar plates and incubated at 20° C. for 24 hours. Colonies of *C. latum* were from 1 to 2 mm. in diameter, tan colored, convex, circular, with undulating edges and with granular glistening surfaces. The organisms were maintained on cow dung extract agar slant at 20° C. and transplanted every seven days. Before testing the action of antiseptics on these organisms they were transplanted three times at 24 hour intervals, two of these transplants were into cow dung extract broth; and the final one on cow dung extract agar, from which the test organisms were obtained.

Part I

Observations With the Compound Microscope

General Methods

Three sets of smears were prepared by pipetting 0.1 ml. of the eight different antiseptic solutions listed under "Materials" on separate clean slides. Employing a platinum needle the test organisms

were removed from the 24-hour old cow dung extract agar culture and mixed in a uniform suspension with the antiseptic under test. Smears of these suspensions were air dried at room temperature (24° C.) and heat fixed. Three sets of controls were made for each of the technics by replacing the respective antiseptics with water in each experiment. The first set of smears was stained with methylene blue to observe the general effect of the antiseptics on the organism. The second set was subjected to hydrolysis and stained with methylene blue to study the effect of the antiseptics on the organism's nuclear materials. The third set was stained with Knaysi's cell-wall stain to study the effect of the antiseptics on the cell wall.

Section A. The Study of the Effect of Antiseptics on C. latum

One set of the smears was stained with 1:100 aqueous solution of methylene blue for 5 minutes, dried and examined under the oil immersion objective.

FINDINGS

1. *Control organisms:*

The test organisms without the antiseptic stained deeply with 1:100 aqueous solution of methylene blue. No cross striations were distinguishable, and although the cell wall was not stained, it appeared as a faint halo-envelope surrounding the organism. Fine granules (nuclear materials), about 0.3 micron in diameter, appeared to be dispersed evenly near the cytoplasmic membrane of the organism as illustrated in figure I.

2. *Organisms treated with resorcinol* (1 per cent aqueous solution):

When the test organisms were treated with resorcinol, the cytoplasm of the organism was not stained with 1:100 aqueous solution of methylene blue. Large granules (about 2 microns in diameter) were arranged in a straight line in the center of the organism as illustrated in figure II.

3. *Organisms treated with mercury bichloride* (1:1,000 aqueous solution):

When the test organisms were treated with mercury bichloride, the cytoplasm of the organisms was disorganized and had a granular (foamy) appearance. Small round granules were present in the organism and no cross striations were visible as illustrated in figure III.

4. *Organisms treated with phenol* (5 per cent aqueous solution):

When the test organisms were treated with 5 per cent phenol, the cytoplasm stained poorly and had a hazy appearance. Striations were not visible. Small and large granules ranging from 1 to 2 microns in diameter were scattered throughout the organism as illustrated in figure IV.

Organisms treated with 1:100 aqueous solution of phenol gave a result similar to those treated with 5 per cent phenol.

5. *Organisms treated with silver nitrate* (1 per cent aqueous solution):

When the test organisms were treated with 1 per cent silver nitrate the cytoplasm stained poorly. The cell wall and the cytoplasmic membrane were affected. There seemed to be no definite outline as compared with the normal organism. Striations remained but faintly stained. A few large granules about 1 to 2 microns in diameter were seen on the cytoplasmic membrane as illustrated in figure V.

6. *Organisms treated with sodium cyanide* (1:1,000 aqueous solution):

When the test organisms were treated with sodium cyanide, the protoplasm aggregated in clumps and gave a foamy appearance. The whole cell appeared as a mass of precipitated protein. There was no definition between the cytoplasmic inclusion and the nuclear materials as illustrated in figure VI.

7. *Organisms treated with sodium arsenite* (1 per cent aqueous solution):

When the test organisms were treated with sodium arsenite, the whole organism stained deeply and large granules were seen in the center of the organism. Striations were present as illustrated in figure VII.

8. *Organisms treated with sodium fluoride* (1 per cent aqueous solution):

When the test organisms were treated with sodium fluoride, the organism as a whole was not affected. It took up less stain as compared with the normal organism, but stained evenly. Striations appeared in some cells, and many shapeless large granules were present on the cytoplasmic membrane as illustrated in figure VIII.

9. *Organisms treated with Lugol's Iodine Solution* (Gram's modification):

When the test organisms were treated with Lugol's Iodine Solution the cytoplasm presented a hazy and granular appearance stained more faintly than the control normal organism. Large granules with a diameter of 1.5 microns were seen in the center of the cell. No striations can be distinguished as illustrated in figure IX.

Discussion and Interpretation of the Results in Part I, Section A, "The Study of the Effect of Antiseptics on C. latum"

1. *Organisms treated with phenol and resorcinol:*

Phenol has long been used as an effective germicidal agent. The germicidal action of phenol was summarized by McCulloch as:

> —"its ability to denature protein. Reichel (1909) postulated phenol to be a protoplasmic poison, the action of which was more physical than chemical, the phenol being able to pass into solution with such substances as coagulated albumin, certain lipoids and the protoplasm of bacteria. Bechhold (1922) maintained that the combination of protein and phenol was an absorption phenomenon. Cooper and Woodhouse (1923) noted a parallelism between the denaturation of protein and the bactericidal actions, and suggested that this may be due to the enlargement of the colloidal units as a result of denaturation and transfer of water to the protein phase, rather than to actual separation of the cell proteins. Schubert and Richter (1925) concluded that the phenols kill by interference with the oxidation-reduction potential equilibrium of the cells. Kojima (1931) demonstrated that the concentrations of phenol which were sufficient to kill bacteria did not coagulate egg albumin and he submitted this as evidence against the theory of direct coagulation of the cell proteins" (22).

It is interesting to note in this investigation that phenol and resorcinol exerted the same action on the cells as shown in figures II, IV, XI, XIII, XX, XXII, XXX, XXXI, XXXVI, XXXVII and XXXVIII. Phenol and resorcinol react with bacterial proteins to form insoluble albuminates. Their abilities to denature the proteins

are in agreement with the absorption phenomenon as proposed by Bechhold (22) and their denaturation is due to the enlargement of the colloidal units as proposed by Cooper and Woodhouse (22). Since the proteins were coagulated in such a way as indicated in the photomicrographs, it is probable that the oxidation-reduction potential equilibrium of the cells were greately disturbed as shown by Schubert and Richter (22).

2. *Organisms subjected to mercury bichloride and sodium arsenite:*

Ehrlich suggested that arsenicals reduce the sulfhydryl groups of the cell (9). Fildes observed the mercurials like arsenicals have a great affinity for sulfhydryl groups thus they preciptate the proteins (11). McCalla (21) has shown that Hg^{++} was strongly absorbed and was toxic in dilute solution.

In this investigation, it was evident that the mercury bichloride and sodium arsenite preciptated the protein and changed the cytoplasm of the organism into a solid, formless mass. This gave the organism a foamy appearance as shown in figures III, VII, XVI, XXI, XXV, XXVII, XXXIV and XL. The nuclear granules (see paragraph under "The study of the effect of antiseptics on the nuclear materials") remained in their normal form and size. From this observation it is suggested that the nucleoproteins of *C. latum* contain no sulfhydryl groups, since they were not affected by these chemicals.

3. *Organisms subjected to silver nitrate:*

Silver nitrate is one of the "heavy metal disinfectants" widely used in disinfection. Its toxic action has been critically investigated by many workers (31) (14) (6). However, the mode of action is not clear at present, probably due to the astringent and corrosive property of the Ag^{+} (28).

In 1918 Matsunaga (20) injected a mixture of silver and copper powder into guinea-pigs and suggested the use of these metallic powders in infected wounds. Tammann (31) found that extremely dilute silver (10^{-3} to 10^{-6}) in oxygenated water was toxic to bacteria. Hasse (14) reported that the effect of silver foil in wounds had no advantage, but that gauze containing silver manganite or silver thiocellobiose was much more powerful than the silver foil. The toxicity due to cations depends on the effect of the cations on the physical properties of a given protein, such as coagulation, solubility, viscosity, etc. (6).

In this investigation, it was found that the silver nitrate aggregates the cytoplasm, thus transferring the proteins into a shapeless mass as shown in figures V, XIV and XXXI. The hazy appearance around the organism is probably due to the astringent action of the Ag^+.

4. *Organisms subjected to sodium cyanide:*

The toxicity of cyanide is generally believed to be caused by its ability to combine with one or more of the respiratory enzymes, thus inhibiting respiration of the organism (3). Cyanides are commonly employed as an insecticidal fumigant. This gas has little or no bactericidal efficiency (13).

In this investigation it was found that the cyanide does affect the protoplasm. The protoplasm of the organism was aggregated and gave a foamy appearance (see figures VI and XXXIII). In this case the cyanide may be combined with the protein to form a protein complex.

5. *Organisms subjected to sodium fluoride:*

The antibacterial action of sodium fluoride may be due to its effect on the oxidation mechanism of the cell (6), and to the oxidizing power of the fluorine ion on some components of the proteins, especially the nucleoproteins, as shown in figures VIII, XVII and XXXV. The nuclear masses were greatly increased in size.

6. *Organisms subjected to Lugol's Iodine Solution* (Gram's Modification):

Iodine has long been used as an efficient germicide. The presence of free iodine in solution has been known to be an active bactericide

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PLATE 1 (*opposite*) illustrates the appearance of *Caryophanon latum*, prepared by suspending the organisms, with or without treatment as indicated, stained with aqueous methylene blue (1:100) solution and observed under a compound microscope (X 1500).

Fig. I. Normal organisms (*C. latum*) prepared by suspending in distilled water.
Fig. II. *C. latum* treated with resorcinol (1 per cent aqueous solution).
Fig. III. *C. latum* treated with mercury bichloride (1:1,000 aqueous solution).
Fig. IV. *C. latum* treated with phenol (5 per cent aqueous solution).
Fig. V. *C. latum* treated with silver nitrate (1 per cent aqueous solution).
Fig. VI. *C. latum* treated with sodium cyanide (1:1,000 aqueous solution).
Fig. VII. *C. latum* treated with sodium arsenite (1 per cent aqueous solution).
Fig. VIII. *C. latum* treated with sodium fluoride (1 per cent aqueous solution).
Fig. IX. *C. latum* treated with Lugol's Iodine Solution (Gram's Modification).

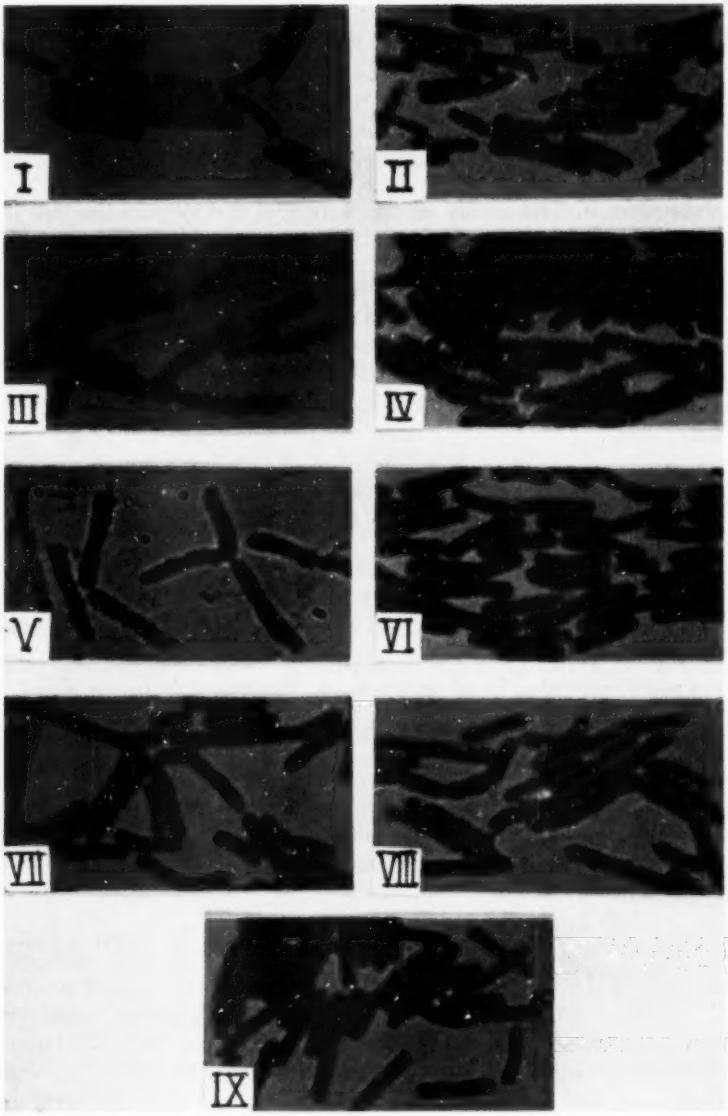

PLATE 1.

(23), and has also been shown to be a viricidal agent (32) (8). Its germicidal action is probably due to its penetrating power (27), its oxidation property, etc.

Evidences obtained from this investigation showed that iodine affects the bacterial proteins. However, the protoplasm and the nucleoprotein were not aggregated as shown in figure IX.

Section B. The Study of the Effect of Antiseptics on the Nuclear Material

See the procedure outlined under "Methods" (see page 7).

The second set of smears was subjected to hydrolysis by placing them in boiling water for 10 minutes. They were then air dried, stained with 1:100 aqueous solution of methylene blue for 5 minutes, rinsed in running water, drained, dried and examined under oil immersion.

Findings

1. *Control organisms:*

The test organisms without the antiseptic when subjected to boiling water for 10 minutes and then stained with methylene blue, revealed that the cytoplasm took no stain at all. Granules on or near the vicinity of the cytoplasmic membrane which took the methylene blue stain were seen in all organisms as illustrated in figure X.

2. *Organisms treated with resorcinol* (1 per cent aqueous solution):

When the test organisms were treated with resorcinol, the cytoplasm was not stained with methylene blue. Large, round granules stained deeply with methylene blue were arranged in an orderly fashion in a straight line in the center of the cell as illustrated in figure XI.

3. *Organisms treated with mercury bichloride* (1:1,000 aqueous solution):

When the test organisms were treated with mercury bichloride, the cytoplasm was slightly stained with methylene blue. Striations were faintly visible. A few granules were attached to the cytoplasmic membrane, but were absent in the center of the organism as illustrated in figure XII.

4. *Organisms treated with phenol* (5 per cent aqueous solution):

When the test organisms were treated with phenol, the cytoplasm was not stained with methylene blue. Many granules having a diameter of 1 micron were scattered throughout the organism and gave a foamy appearance as illustrated in figure XIII.

Phenol, 1:100 aqueous solution, presented a similar picture.

5. *Organisms treated with silver nitrate* (1 per cent aqueous solution):

When the test organisms were treated with silver nitrate, the cytoplasm was not stained with methylene blue. Nuclear granules attached to the cytoplasmic membrane were seen. The organism had a foamy appearance as illustrated in figure XIV.

6. *Organisms treated with sodium cyanide* (1:1,000 aqueous solution):

When the test organisms were treated with sodium cyanide, the cytoplasm was not stained with the methylene blue. A few nuclear granules stained with methylene blue solution were seen attached to the cytoplasmic membrane. The cross striations of the organism could be recognized, although they were not stained with the dye. See figure XV.

7. *Organisms treated with sodium arsenite* (1 per cent aqueous solution):

When the test organisms were treated with sodium arsenite, the cytoplasm was not stained with the methylene blue solution. Nuclear granules attached to the cytoplasmic membrane were seen. The organism had a foamy appearance as illustrated in figure XVI.

8. *Organisms treated with sodium fluoride* (1 per cent aqueous solution):

When the test organisms were treated with sodium fluoride, the cytoplasm was not stained with methylene blue solution. Striations were seen, and internal granules were present in the center of the organism as shown in figure XVII.

9. *Organisms treated with Lugol's Iodine solution* (Gram's Modification):

When the test organisms were treated with Lugol's Iodine Solution, the cytoplasm was not stained with methylene blue. Large

granules having a diameter ranging from 1 to 1.5 microns were seen in the center of the organism. A few organisms had no granules at all as shown in figure XVIII.

Interpretation and Discussion of Results of Part I, Section B, "The Study of the Effect of Antiseptics on the Nuclear Material"

C. latum stained deeply with methylene blue dye. This may be due to a high ribonucleic acid content in the cytoplasm and in the cytoplasmic membrane of the organism as suggested by Knaysi (18):

> "The cytoplasmic membrane is hyperchromatic, staining deeply with basic and neutral dyes over a wide range of pH.—the cytoplasm is strongly acidic (i.e., basophilic) because of its high ribonucleic acid content,—the acidic strength of the cytoplasmic membrane is usually surpassed only by that of volutin; in certain bacteria, it may be equaled by that of the nucleus."

In order to study the nuclear changes after the organism has been subjected to certain antiseptics, the volutin material must be removed by hydrolysis as in Feulgen test for nuclear reaction, or by hydrolysis in boiling water with or without acid for 10 minutes and stained with a basic or a neutral dye.

1. *Control organisms:*

C. latum was subjected to hydrolysis in boiling water for 10 minutes and stained with 1:100 aqueous solution of methylene blue. The cytoplasm is not stained with the dye indicating that the volutin material is removed. The large granules attached to the cytoplasmic

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PLATE 2 (*opposite*) illustrates the appearance of *Caryophanon latum*, with or without treatment as indicated, subjected to hydrolysis, stained with aqueous methylene blue (1:100) solution and observed under a compound microscope (X 1500).

Fig. X. Control organisms (*C. latum*).
Fig. XI. *C. latum* treated with resorcinol (1 per cent aqueous solution).
Fig. XII. *C. latum* treated with mercury bichloride (1:1000 aqueous solution).
Fig. XIII. *C. latum* treated with phenol (5 per cent aqueous solution).
Fig. XIV. *C. latum* treated with silver nitrate (1 per cent aqueous solution).
Fig. XV. *C. latum* treated with sodium cyanide (1:1000 aqueous solution).
Fig. XVI. *C. latum* treated with sodium arsenite (1 per cent aqueous solution).
Fig. XVII. *C. latum* treated with sodium fluoride (1 per cent aqueous solution).
Fig. XVIII. *C. latum* treated with Lugol's Iodine Solution (Gram's Modification).

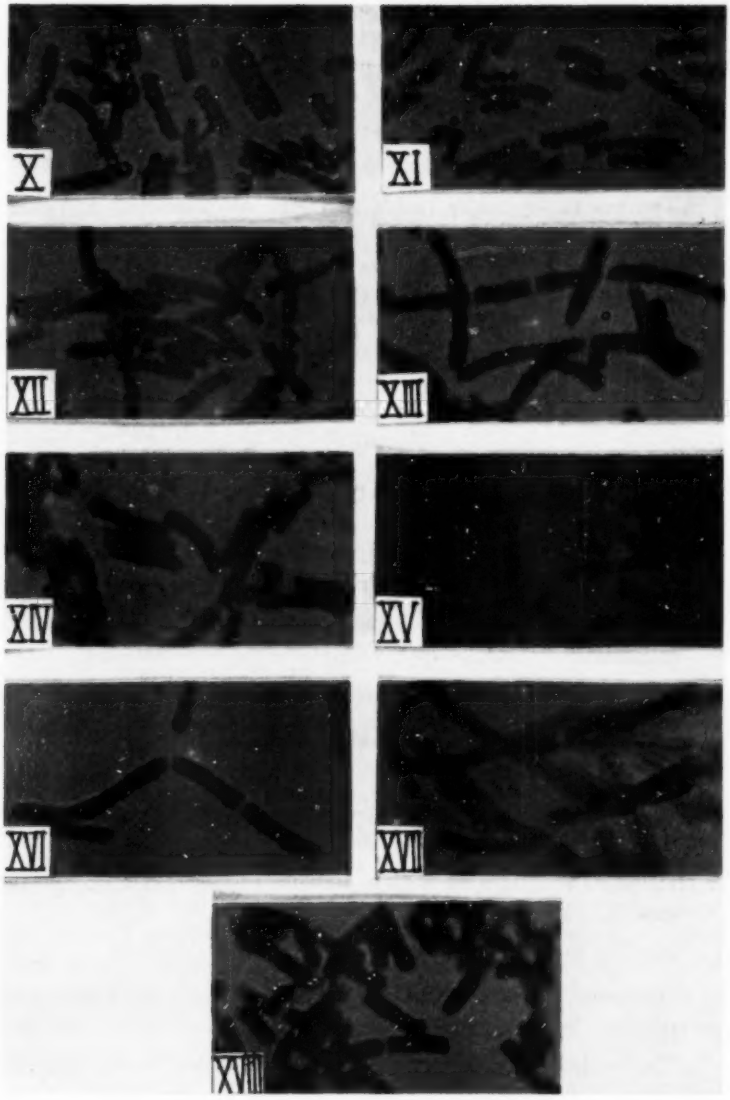

PLATE 2.

membrane were stained blue. These granules were assumed to be desoxyribonucleic acid, and this supposition was confirmed by a positive Feulgen reaction (12). The nuclear granules in the normal control organisms having a diameter of 1 micron adhered to the cytoplasmic membrane as shown in figure X.

2. *Organisms treated with resorcinol* (1 per cent aqueous solution):

The nuclear granules had a diameter of 2 microns and were orderly arranged in the center of the organism in a straight line as shown in figure XI. Resorcinol seemed to have a great affinity for the nuclear materials and aggregated this material in a large mass.

3. *Organisms treated with mercury bichloride* (1:1,000 aqueous solution):

Nuclear materials were not affected by mercury bichloride.

4. *Organisms treated with phenol* (5 per cent aqueous solution):

Many granules had a diameter of 1 micron and were attached to the cytoplasmic membrane and scattered throughout the organism. It seemed that phenol probably precipitated some volutin material along with proteins and thus could not be hydrolyzed.

5. *Organisms treated with silver nitrate* (1 per cent aqueous solution):

The nuclear material was not affected by silver nitrate.

6. *Organisms treated with sodium cyanide* (1:1,000 aqueous solution):

The nuclear material was apparently affected by cyanide. There were fewer granules as compared with the normal control smear.

7. *Organisms treated with sodium arsenite* (1 per cent aqueous solution):

Nuclear material was not affected by sodium arsenite.

8. *Organisms treated with sodium fluoride* (1 per cent aqueous solution):

The nuclear granules were removed from their normal positions (adhering to the cytoplasmic membrane) and rearranged in the center of the organism.

9. *Organisms treated with Lugol's Iodine Solution* (Gram's Modification):

The nuclear granules were removed from their normal positions, the cytoplasmic membrane, and rearranged in the center of the organism as shown in sodium fluoride treatment, with the exception that these were much larger in size.

Section C. The Study of the Effect of Antiseptics on the Cell Wall

Following the procedure outlined under "Methods" (page 7), the third set of smears were stained with Knaysi's cell-wall stain (19), dried and examined with the compound microscope under oil immersion.

Findings

1. *Control organisms:*

The cell wall and striations (cross-walls) were clearly stained as illustrated in figure XIX.

2. *Organisms treated with resorcinol* (1 per cent aqueous solution):

The cell wall appeared normal. Striations (cross-walls) were not clearly stained. They seemed somewhat altered by the resorcinol as demonstrated in figure XX.

3. *Organisms treated with mercury bichloride* (1:1,000 aqueous solution):

The cell wall appeared normal. Striations (cross-walls) stained faintly and appeared as thin, fine threads as illustrated in figure XXI.

4. *Organisms treated with phenol* (5 per cent aqueous solution):

When the test organisms were treated with 5 per cent phenol, the cell wall was badly disorganized. Some parts along the cell wall were greatly reduced in thickness, and it seemed to be lysed by the 5 per cent phenol. Cross striations were clearly visible. The organism was altered from its normal regular form and appeared shriveled as illustrated in figure XXII.

When the test organisms were treated with 1:100 aqueous solution of phenol, the cell wall presented a similar picture, except that the effect on the striations was lessened.

5. *Organisms treated with silver nitrate* (1 per cent aqueous solution):

When the test organisms were treated with silver nitrate, the cell wall appeared normal. The striations (cross-walls) stained clearly and were distinctly visible. However, it gave a somewhat disorganized appearance as shown in figure XXIII.

6. *Organisms treated with sodium cyanide* (1:1,000 aqueous solution):

When the test organisms were treated with sodium cyanide, the cell wall was not affected by this chemical. The cell wall appeared normal. Striations (cross-walls) were clearly seen and stained evenly as shown in figure XXIV.

7. *Organisms treated with sodium arsenite* (1 per cent aqueous solution):

When the test organisms were treated with sodium arsenite, the cell wall appeared normal. The striations (cross-walls) were not well stained. They were, however, visible as shown in figure XXV.

8. *Organisms treated with sodium fluoride* (1 per cent aqueous solution):

When the test organisms were treated with sodium fluoride, the cell wall appeared normal. Striations (cross-walls) in some organisms gave an abnormal impression and irregularly spaced as compared with the control organisms as shown in figure XXVI.

PLATE 3 (*opposite*) illustrates the appearance of *Caryophanon latum*, with or without treatment as indicated, stained with Knaysi's cell-wall stain and observed under a compound microscope (X 1500).

Fig. XIX. Control organisms (*C. latum*).
Fig. XX. *C. latum* treated with resorcinol (1 per cent aqueous solution).
Fig. XXI. *C. latum* treated with mercury bichloride (1:1000 aqueous solution).
Fig. XXII. *C. latum* treated with phenol (5 per cent aqueous solution).
Fig. XXIII. *C. latum* treated with silver nitrate (1 per cent aqueous solution).
Fig. XXIV. *C. latum* treated with sodium cyanide (1:1000 aqueous solution).
Fig. XXV. *C. latum* treated with sodium arsenite (1 per cent aqueous solution).
Fig. XXVI. *C. latum* treated with sodium fluoride (1 per cent aqueous solution).
Fig. XXVII. *C. latum* treated with Lugol's Iodine Solution (Gram's Modification). The right corner of the photograph was out of focus. Organisms shown by arrows were in focus.

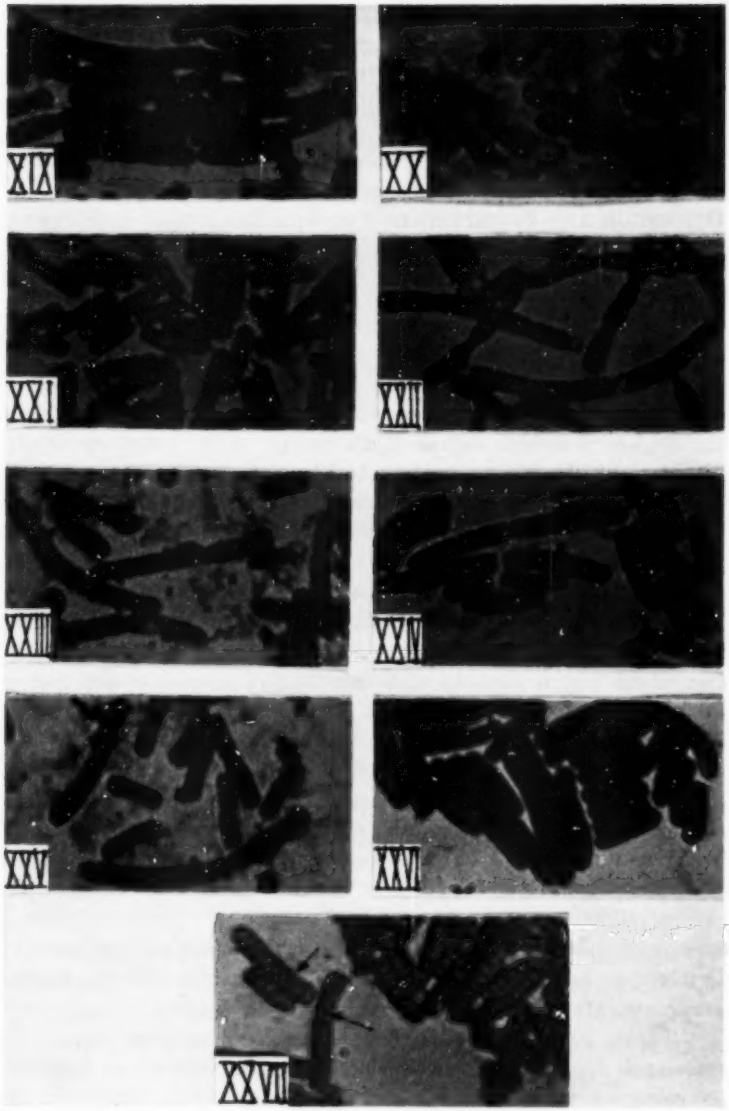

PLATE 3.

9. *Organisms treated with Lugol's Iodine Solution* (Gram's Modification):

When the test organisms were treated with Lugol's Iodine Solution the cell wall appeared normal. The striations (cross-walls) stained clearly, showing slight thickening at the center and tipping to a fine point at their ends as shown in figure XXVII by arrows.

Discussion and Interpretation of the Results of Experiment Part I, Section C, "The Study of the Effect of the Antiseptics on the Cell Wall"

In 1870, Cohn suspected the existence of a cell wall by the evidence that the bacterial cell was resistant to dissolution by treatment with alkalis and acids. As the evidence accumulated, the existence of a cell wall has been proved (26) (18) (17). "The cell wall is endowed with a high degree of elasticity, ductility, and rigidity, as demonstrated by microdissection and by direct observation of living organisms" (15). The chemical composition of the cell wall, however, has not been well established. It has been shown that the cell wall can not be readily stained with any dyes, and in order to stain the cell wall a mordant must be used as in Knaysi's cell-wall staining technic (19). The cell wall has been shown to possess a "—great resistance to chemical treatment (except to strong acids and alkalis) suggesting that it consists of a non-reactive substance and that its function is chiefly concerned with mechanical protection of the cell constituents" (7).

This investigation revealed that when *C. latum* was subjected to 5 per cent aqueous solution of phenol, the cell wall was greately disorganized, and appeared partly disintegrated as shown in figures XXII and XXXVI. The cell wall, however, did not appear to be affected by resorcinol (1 per cent aqueous solution). When 1:100 concentration of phenol was used, the effect was similar to that of 5 per cent solution except that the degree of effect on the striations appeared to be lessened. The other antiseptics used in this investigation appeared to have no effects on the cell wall.

The striations appeared to be affected by aqueous solutions of resorcinol, 1 per cent; mercury bichloride, 1:1,000; silver nitrate, 1 per cent; sodium arsenite, 1 per cent; sodium fluoride, 1 per cent and Lugol's Iodine Solution (Gram's modification). This may have

been due to the fact that the cell wall is more rigid and more resistant than the "cross-walls".

Part II
Observations With the Phase Microscope to Study the Effect of Antiseptics

Materials

A. *Organism: Caryophanon latum* test organism (for preparation see "Isolation of organism.").

B. *Antiseptics:* Described previously.

Methods

The test organisms were suspended in various antiseptic solutions as described under "Materials", and smears were prepared on a series of slides. The wet smears were covered with cover-slips and 20 minutes from the time of mixing the antiseptic with the test organism, they were examined under oil immersion employing a B-minus contrast objective.

Controls were made by suspending the test organisms in isotonic solution of sodium chloride on slides, covered with cover-slips, and examined.

Findings

1. *Control organisms:*

The cellular structures of control organisms observed under a phase microscope were clearly revealed. The striations in each trichome were easily recognized and the internal granules could be readily seen on or closely attached to the cytoplasmic membrane, as shown in figure XXVIII.

2. *Organisms treated with resorcinol* (1 per cent aqueous solution):

When the test organisms were treated with resorcinol, the external structures of the organism presented a picture similar to that of the control organisms as shown in figure XXVIII. However, the internal granules were more conspicuous in size and in numbers as compared with the control organisms. These granules were not orderly arranged as in figure II and in figure XI, but adhered to the vicinity of the cytoplasmic membrane.

3. *Organisms treated with mercury bichloride* (1:1,000 aqueous solution):

When the test organisms were treated with mercury bichloride, the outline of the organism was altered somewhat by the chemical as shown in figure XXIX. Some striations were thicker than the others. No conspicuous granules were seen.

4. *Organisms treated with phenol* (5 per cent aqueous solution):

When the test organisms were treated with 5 per cent aqueous phenol, the striations seemed withdrawn from the cell wall and separated into thick dark and light bands. The round granules normally seen in the organism were absent as shown in figure XXX.

When the test organisms were treated with 1:100 aqueous solution of phenol, the organism was not so markedly changed as in 5 per cent phenol; few thick striations were seen and many round internal granules were present as shown in figure XXXI.

5. *Organisms treated with silver nitrate* (1 per cent aqueous solution):

When the test organisms were treated with silver nitrate, the organism was more transparent than the normal organism. Many

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PLATE 4 (*opposite*) illustrates the appearance of *Caryophanon latum*, with or without treatment as indicated and examined microscopically. Figures XXVIII to XXXV inclusive are as observed under the phase microscope (X 1700). Figures XXXVI to XL inclusive are as shown under the dark field (X 1700).

Fig. XXVIII. Normal control (*C. latum*).
Fig. XXIX. *C. latum* treated with mercury bichloride (1:1000 aqueous solution).
Fig. XXX. *C. latum* treated with phenol (5 per cent aqueous solution).
Fig. XXXI. *C. latum* treated with phenol (1:100 aqueous solution).
Fig. XXXII. *C. latum* treated with silver nitrate (1 per cent aqueous solution).
Fig. XXXIII. *C. latum* treated with sodium cyanide (1:1000 aqueous solution).
Fig. XXXIV. *C. latum* treated with sodium arsenite (1 per cent aqueous solution).
Fig. XXXV. *C. latum* treated with sodium fluoride (1 per cent aqueous solution).
Fig. XXXVI. *C. latum* treated with resorcinol (1 per cent aqueous solution).
Fig. XXXVII. *C. latum* treated with phenol (5 per cent aqueous solution).
Fig. XXXVIII. *C. latum* treated with phenol (1:100 aqueous solution).
Fig. XXXIX. *C. latum* treated with sodium cyanide (1:1000 aqueous solution).
Fig. XL. *C. latum* treated with sodium arsenite (1 per cent aqueous solution).

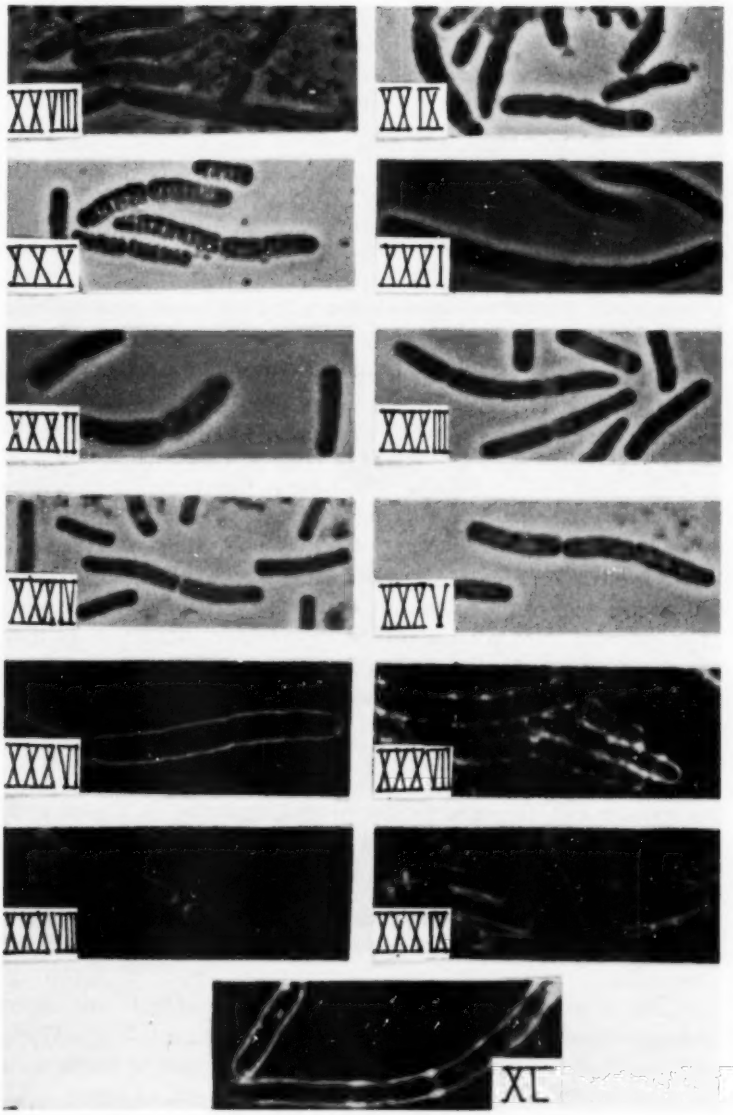
XXVIII
XXIX
XXX
XXXI
XXXII
XXXIII
XXXIV
XXXV
XXXVI
XXXVII
XXXVIII
XXXIX
XL

PLATE 4.

granules were seen on or near the vicinity of the cytoplasmic membrane as shown in figure XXXII.

6. *Organisms treated with sodium cyanide* (1:1,000 aqueous solution):

When the test organisms were treated with sodium cyanide, the organisms lacked morphological definition as observed under the phase microscope. Striations were not clearly visible as in the normal organism. Very few granules were present as shown in figure XXXIII.

7. *Organisms treated with sodium arsenite* (1 per cent aqueous solution):

When the test organisms were treated with sodium arsenite, no round granules as presented in normal organism were seen. The striations were more conspicuous as compared to the normal organism and separated into dark and light bands as shown in figure XXXIV.

8. *Organisms treated with sodium fluoride* (1 per cent aqueous solution):

When the test organisms were treated with sodium fluoride, more than 85 per cent of the organisms showed no striations at all. Many large granules were present on the cytoplasmic membrane in these organisms. The other 15 per cent of the organisms had no internal granules of any kind, but striations were present as in the normal organism. It is possible that this 15 per cent of the organisms were dead. See figure XXXV.

Discussion and Interpretation of the Results of Experiment Part II, "The Effect of Antiseptics as Observed Under the Phase Microscope"

Since the development of the phase microscope, many possibilities and visions have been opened in the field of cytology (2) (5) (16) (30).

It is interesting to see that the results obtained from this experiment agreed rather closely with the findings in section "A". Phenol and resorcinol appeared to precipitate the protoplasmic proteins and the nucleoproteins forming a large mass as shown in figure XXX. Mercury bichloride and sodium arsenite seem to exert their actions

by combining with the sulfhydryl groups of the protein (9) probably causing the precipitation of proteins which were observed within the organisms as shown in figures XXIX and XXXIV. Silver nitrate appeared to coagulate proteins and to precipitate them on the cell wall as shown in figure XXXII. Sodium cyanide probably combined with the proteins of the organism thus forming a viscous complex as shown in figure XXXIII. Sodium fluoride is believed to combine with the protoplasmic proteins and also to form a viscous colloidal matter, thus causing the nuclear granules to aggregate into larger particles as shown in figure XXXV.

The pictures of the organisms observed under the phase microscope are slightly different from those seen under the compound microscope. This difference may have been due to the fact that the organisms observed under the phase microscope were in a wet state. The presence of water rendered the organism more transparent, although the proteins were precipitated. The large dark and light bands appearing in the organism treated with phenol (5 per cent solution) and mercury bichloride could probably be explained as proposed by Cooper and Woodhouse (28) as being due to the retention of water by the denatured proteins which thus enlarge the colloidal units.

Part III

Observations With the Dark-Field Microscope to Study the Effect of Antiseptics on the Cell Wall

Materials

A. *Organism: Caryophanon latum* test organism (for preparation see "Isolation of organism").

B. *Antiseptics:* 1. Phenol, aqueous solutions of 1:20 and 1:100 concentrations. 2. Resorcinol, 1 per cent aqueous solution. 3. Sodium arsenite, 1 per cent aqueous solution. 4. Sodium cyanide, 1:1,000 aqueous solution.

Methods

The test organism was suspended in various antiseptic solutions as described in Part II. A series of smears were prepared on glass slides, covered with cover-slips, and examined 20 minutes from the time of mixing the antiseptic with the test organism under the dark-field microscope.

Findings

1. *Control organisms:*

The cell wall was brightly and evenly illuminated and had a thickness of 0.3 micron (actual measurement with a micrometer). The striations were evenly spaced. Internal granules were seen.

2. *Organisms treated with resorcinol* (1 per cent aqueous solution):

When the test organisms were treated with resorcinol, the cell wall was not affected; striations (cross-walls) were absent. Large internal granules were scattered throughout the organism as shown in figure XXXVI.

3. *Organisms treated with phenol* (5 per cent aqueous solution):

When the test organisms were treated with 5 per cent phenol, the cell wall was badly disorganized. Swellings appeared along the periphery of the cell wall. Large, shapeless granules were seen throughout the cell. See figure XXXVII.

4. *Organisms treated with 1:100 aqueous solution of phenol:*

When the test organisms were treated with 1:100 phenol the cell wall appeared normal. Few striations (cross-walls) remained. Large internal granules were seen to be orderly arranged in the center of the organisms. They were not badly affected as in 5 per cent phenol. See figure XXXVIII.

5. *Organisms treated with sodium arsenite* (1 per cent aqueous solution):

When the organisms were treated with sodium arsenite the cell wall was not affected; it appeared normal. Granules were seen in the organism as shown in figure XXXX.

Discussion and Interpretation of the Results of Experiment Part III, "The Effect of Antiseptics as Observed Under the Dark-Field Microscope"

The cell wall and the intracellular granules of *C. latum* could be readily revealed with the dark-field technique. The cytoplasm appeared black, the intracellular granules bright and the whole organism

was surrounded by a bright line. This bright line corresponds to the cytoplasmic membrane and the cell wall.

This investigation was designed to show which antiseptics affected the cell wall and to what degree. The cell wall possesses a great resistance to chemical treatment, and appeared to be affected only by strong acids and alkalis (*7*). When the test organisms were subjected to 5 per cent aqueous solution of phenol, the cell wall was badly disorganized as shown in figure XXXVII. When 1:100 concentration of phenol was used on the organism, the effect was lessened as shown in figure XXXVIII. The test organisms were subjected to the other antiseptics such as 1:1,000 sodium cyanide and 1 per cent sodium arsenite and observed under the dark-field microscope. There was no evidence that the cell wall had been affected by these antiseptics.

Part IV
Plasmolysis and Plasmoptysis

MATERIALS:

A. *Organism: Caryophanon latum* test organism (for preparation see "Isolation of organism").

B. *Sodium chloride,* 30 per cent aqueous solution.

C. *Surface active agents:*

1. "Tergitol" Penetrant 4, 1:100 aqueous solution.
2. Tetrosan 60%, 1:100 aqueous solution.

METHODS:

1. The test organisms were removed from an agar slant with inoculating needle, were subjected to plasmolysis by suspending them in 0.2 ml. of 30 per cent sodium chloride solution on a slide. The slide was covered with a petrolatum ringed cover-slip to prevent evaporation. Observations were made under the phase microscope at 10 minute intervals for 2 hours.

2. The test organisms removed from an agar slant with inoculating needle, were subjected to plasmoptysis by suspending them in distilled water on a slide. The slide was covered with a petrolatum ringed cover-slip to prevent evaporation, and examined under the

phase microscope after 30 minutes, 1 hour and 2 hours from the time of suspending the organisms in distilled water.

3. The test organisms were removed from an agar slant, placed in 0.2 ml. of each of the solutions of surface active agents, as listed under "Materials", on a hanging drop slide. They were allowed to stand for 20 minutes. Distilled water (0.4 ml.) was then added and the slide was covered with a petrolatum ringed cover-slip to prevent evaporation. It was then examined under the phase microscope at 10 minute intervals for 2 hours.

Findings:

1. The result in plasmolysis when the test organisms were subjected to 30 per cent sodium chloride was positive.

2. When the test organisms were suspended in distilled water for two hours, and in the presence of the surface active agents, plasmoptysis was not observed.

Discussion and Interpretation of the Results

Plasmolysis of the organisms was evident by the fact that two adjacent "cross-walls" were drawn toward each other and formed a biconcave lens. However, the cytoplasmic membrane of the organisms was not withdrawn from the cell wall. This peculiar phenomenon may be explained by the architectural structures of the organism. The "cross-walls" support the cell wall and increase its resistance toward any external physical force when subjected to the organism. This "reenforcement" of the organism to external forces may also be responsible for the negative results in plasmoptysis. The organisms become more turgid than the normal control organisms, but have never been observed to reach the point of rupture even when treated with surface active agents. It has also been observed that the dead cells (showing no growth on subculture) remain intact in their normal forms in a three months' old or older broth cultures. This may probably be due to the fact that the cell wall is composed of some resistant materials or that this organism possesses no autolytic enzymes.

Part V
Observations Suggesting That C. latum Is a Multicellular Organism

Materials

1. Mortar containing fine sand and pestle was wrapped with paper and sterilized in dry air oven (4 hours at 170° C.).

2. *Media:* Cow dung extract broth and agar.

3. *Pipettes:* Sterilized in dry air oven (2 hours at 170° C.).

Method

C. latum was introduced into a sterile mortar containing sterile fine sand by a sterile pipette. The organisms were ground with a sterile pestle. Two 4 mm. loopsful of the supernatant were transferred on a slide periodically and examined under a microscope until the normal form of the organism could not be recognized. Two 4 mm. loopsful of the supernatant were transferred to cow dung extract broth and agar media and incubated at 20° C. for 16 hours.

Findings

The organisms resumed their normal size and morphology after incubation at 20° C. for 16 hours.

Discussion and Interpretation of Results

When *C. latum* was observed under a compound or a phase microscope the organism gave the appearance of alternating light and dark striations in each trichome. This trichome appeared chambered and contained many cells (29). Weeks and Kelley have pointed out that this trichome is not a coenocytic unit: "The coenocytic-like condition may be explained as the result of rapid growth and delayed crosswall formation" (33) (see figure XXXVI).

In this investigation the organisms were fragmented beyond recognition by prolonged grinding until no appreciable portions of the organism could be recognized under a compound microscope. When two 4 mm. loopsful of the supernatent were transferred into cow dung extract broth, the organisms resumed their normal size and morphology in 16 hours of incubation. This would indicate that the

normal forms might have developed from parts of the organism i.e. that *C. latum* might be a multicellular organism. This supposition is strengthened by the observations on plasmolysis in experiment "Part IV", and could be seen under the phase microscope when the organisms were subjected to 5 per cent phenol as shown in figure XXX and when treated with sodium arsenite as in figure XXXIV.

Summary

1. *Caryophanon latum* was isolated from cow dung, and the stock culture was maintained on cow dung extract agar slants. The test organisms were obtained by transferring from the stock culture three times on three consecutive days, two of these transfers were into cow dung extract broth and the final one on cow dung extract agar.

2. *C. latum* was subjected to the action of aqueous solutions of eight antiseptic solutions: 1. phenol, 5 per cent; 2. resorcinol, 1 per cent; 3. mercury bichloride, 1:1,000; 4. sodium arsenite, 1 per cent; 5. silver nitrate, 1 per cent; 6. sodium fluoride, 1 per cent; 7, sodium cyanide, 1:1,000 and 8. Lugol's Iodine Solution (Gram's Modification).

3. Three sets of slides were prepared from the above mixtures, air dried and fixed by heat.

A. The first set of slides was stained with 1:100 aqueous solution of methylene blue to observe the general effect of the antiseptics on the organism using a compound microscope.

B. The second set of slides was subjected to hydrolysis in boiling water for 10 minutes, then stained with 1:100 aqueous methylene blue solution. Observations were made using the oil immersion lens to study the effect of the antiseptics on the organism's nuclear materials.

C. The third set of slides was stained with Knaysi's cell-wall stain to study the effect of the antiseptics on the cell wall. Observations were made by using a compound microscope.

4. *C. latum* was suspended in the eight antiseptic solutions (see under 2). Wet smears were examined under the phase microscope to study the general effect of the antiseptics on the organism.

5. Smears were made as in phase contrast observations. The smears were observed under darkfield illumination to study the effect of antiseptics on the cell wall.

6. *C. latum* was subjected to plasmolysis and plasmoptysis to study the resistance of the cell wall and the structure of *C. latum*.

7. *C. latum* was ground in mortar and pestle with fine sand and this fragmented organism was transferred to cow dung extract broth medium to observe whether or not *C. latum* is a coenocytic organism.

Conclusions

1. Phenol, 1 per cent and 5 per cent aqueous solutions and resorcinol, 1 per cent aqueous solution appeared to react with bacterial proteins to form insoluble proteinates. The denaturation of proteins by phenol and resorcinol may be due to the enlargement of the colloidal units, as proposed by Cooper and Woodhouse (*22*).

2. Mercury bichloride of 1:1,000 concentration of aqueous solution and sodium arsenite of 1 per cent aqueous solution did not affect the nuclear materials of *C. latum*. This may possibly be due to the fact that the nucleoproteins of *C. latum* contains no sulfhydryl groups.

3. Silver nitrate (1 per cent aqueous solution) appeared to exert its germicidal action by its astringent property.

4. Sodium cyanide (1:1,000 aqueous solution) combined with proteins to form a viscous mass.

5. Sodium fluoride (1 per cent aqueous solution) seemed to possess more affinity for the nuclear materials.

6. Lugol's Iodine Solution (Gram's Modification) appeared to precipitate the bacterial proteins.

7. When the organisms were subjected to boiling water for 10 minutes, all volutin materials were hydrolyzed; and the nuclear materials (granules) could easily be studied by staining with a basic dye.

8. Only 5 per cent phenol affected the cell wall of *C. latum*, and it appeared partly disintegrated and badly disorganized.

9. Observations with the phase and darkfield microscopes were similar to that observed with the compound microscope.

10. From the results obtained by plasmolysis, plasmoptysis and grinding the organism with fine sand, as in experiments IV and V, it appears that *C. latum* is a multicellular organism.

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BOOK REVIEWS

An Introduction to the Chemistry of the Hydrides. By Dallas T. Hurd. x + 231 pages. John Wiley and Sons, Inc., 440 Fourth Avenue, New York 16, N. Y. 1952.

The writer of this excellent book has for the first time brought together a coordinated presentation of the theory, the properties and the reactions of the hydrides. Chapter one deals with the term "hydride" and types in relation to the periodic table. The second chapter gives an excellent exposition on the chemical bonding of hydrogen.

In this volume the hydrides are classified for purposes of discussion and description, as ionic hydrides, transitional metal hydrides, borderline hydrides, covalent hydrides, and complex hydrides. Many references are given to books and periodicals for those who are interested in this fast growing field of chemistry.

The author feels that there are certain inconsistencies in nomenclature of the hydrides (chapter 17) and suggests that systematic methods of naming should be established before too many new compounds appear. The latter part of the book deals with deuterides, toxicology of hydrides, vacuum manipulation of volatile hydrides, and firms from which hydrides can be procured. An author index is included. Anyone interested in this field will find that this readable, compact volume is a very worthwhile addition to his library.

C. C. PINES

Anatomy and Physiology Laboratory Manual. By Verna March Vogel, B.S., M.A., Jesse D. Perkinson, Jr., B.S., M.S., Ph.D. The C. V. Mosby Co., St. Louis, 1949.

The chief purpose of a student manual is to help the student to better understand the subject. The *Anatomy and Physiology Laboratory Manual* by *Vogel and Perkinson* adequately covers the major systems of the body. Both the gross and microscopic anatomy

are reviewed with exercises and illustration of the various physiology functions.

The manual would benefit by having lighter shading in the illustrations from page 42 to 53. Most of the important details of the bone structure here are lost. It would probably help coordinate the subject matter to see the bones in anatomic relationship to the rest of the body. Perhaps as a sketchy outline as seen on page 62. The addition of a skeleton of the body to show all the boney structure in their proper position would add considerably.

The vocabulary guide which is at the beginning of each unit serves as a good introductory basis to better understand the subject to follow. The manual should be used in conjunction with any standard textbook of anatomy and physiology.

If all the units are followed as outlined, the student would systematically learn a great deal of anatomy and physiology as presented in this manual.

J. I. FEINMAN, Ph.G., M.D.

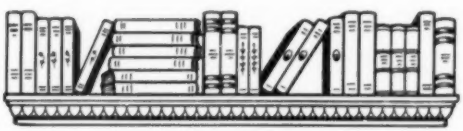
The Production of Antibodies. (Monograph of the Walter and Eliza Hall Institute, Melbourne, Australia). By F. M. Burnet and Frank Fenner; Macmillan and Company, Ltd., 1949. $3.00.

This book is very unusual in several respects. It covers the subject very well as evident from the chapter titles.—

Chapter I. The General Character of Antibodies
Chapter II. The Functions of Plasma Proteins
Chapter III. The Rise and Fall of Antibody Titre in the Blood of Actively and Passively Immunized Animals
Chapter IV. Qualitative Differences amongst Antibody Molecules
Chapter V. The Site of Antibody Production
Chapter VI. The Evidence that Antibody Production May Continue in the Absence of Antigen
Chapter VII. Immunological Behaviour of Young Animals
Chapter VIII. Theoretical Aspects of Antibody Production
Chapter IX. Antibodies Involved in Sensitization Phenomena
Chapter X. Tissue Transplantation Immunity in Relation to Immunological Theory
Chapter XI. Summary and Conclusions

Although the book is technical and scientific it is written with a gentle hand that leads the reader confidently through its labyrinth of data and facts without the usual aloofness and coldness of similar books on such material. The authors frankly admit that they do not know all the answers, which is a refreshing approach. The book has more the quality of a series of informal lectures followed by a discussion period with questions and answers and some unanswered questions. It is a thoughtfully revised edition of an earlier monograph with portions eliminated and other parts enlarged wherever new facts have changed the story of antibodies in the intervening years. For example, there is newer material on the genetic control of enzymes and antigens and the process of protein synthesis in the cell. There is also the change of emphasis from reticuloendothelial cells to lymphocytes and plasma cells as the probable producers of antibodies. The chief novelty of the authors' presentation is the introduction of the "self-maker" hypothesis. The authors attempt to shake loose the binding of immunological thought on antibodies from the restrictive possession of the various biological subsciences and place it more in the main stream of biological discovery and generalization. Anybody interested in biological phenomena (not only bacteriologists, as there are special references of interest to embryologists as well as parasitologists) cannot help but feel refreshed after reading this book, for the reader has had revealed for him a science still young in thought and one needing his personal attention. It is a challenge and the reader wants to leave his armchair complacency and rush to the laboratory and start experimenting.

FRANCIS M. WHITE



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